

## US-102

```
SEQUENCE LISTING
<110> Ajinomoto Co. Inc.
<120> Method for Producing L-Amino Acid Using Methylotroph
<130> OP1627/US-102
<140>
<141> 2003-11-
<150> JP 2002-336315
<151> 2002-11-20
<160> 14
<170> PatentIn Ver. 2.0
<210> 1
<211> 711
<212> DNA
<213> Brevibacterium lactofermentum
<220>
<221> CDS
<222> (1)..(711)
<400> 1
atg gtg atc atg gaa atc ttc att aca ggt ctg ctt ttg ggg gcc agt
Met Val Ile Met Glu Ile Phe Ile Thr Gly Leu Leu Gly Ala Ser
                                                                                 48
                                             10
ctt tta ctg tcc atc gga ccg cag aat gta ctg gtg att aaa caa gga
Leu Leu Leu Ser Ile Gly Pro Gln Asn Val Leu Val Ile Lys Gln Gly
                                        25
                                                                 30
                                                                                 144
att aag cgc gaa gga ctc att gcg gtt ctt ctc gtg tgt tta att tct
Ile Lys Arg Glu Gly Leu Ile Āļā Val Leu Leu Val Cys Leu Ile Ser
           35
                                   40
                                                            45
gac gtc ttt ttg ttc atc gcc ggc acc ttg ggc gtt gat ctt ttg tcc
Asp Val Phe Leu Phe Ile Ala Gly Thr Leu Gly Val Asp Leu Leu Ser
                                                                                 192
     50
                              55
                                                       60
                                                                                 240
aat gcc gcg ccg atc gtg ctc gat att atg cgc tgg ggt ggc atc gct
Asn Ala Ala Pro Ile Val Leu Asp Ile Met Arg Trp Gly Gly Ile Ala
                                                                                 288
tac ctg tta tgg ttt gcc gtc atg gca gcg aaa gac gcc atg aca aac
Tyr Leu Leu Trp Phe Ala Val Met Ala Ala Lys Asp Ala Met Thr Asn
aag gtg gaa gcg cca cag atc att gaa gaa aca gaa cca acc gtg ccc
Lys Val Glu Ala Pro Gln Ile Ile Glu Glu Thr Glu Pro Thr Val Pro
                                                                                 336
                                       105
              100
                                                                                 384
gat gac acg cct ttg ggc ggt tcg gcg gtg gcc act gac acg cgc aac
Asp Asp Thr Pro Leu Gly Gly Ser Ala Val Ala Thr Asp Thr Arg Asn
                                                           125
                                  120
         115
cgg gtg cgg gtg gag gtg agc gtc gat aag cag cgg gtt tgg gta aag
Arg Val Arg Val Glu Val Ser Val Asp Lys Gln Arg Val Trp Val Lys
                                                                                 432
     130
                             135
                                                      140
```

Page 1

```
US-102
ccc atg ttg atg gca atc gtg ctg acc tgg ttg aac ccg aat gcg tat
Pro Met Leu Met Ala Ile Val Leu Thr Trp Leu Asn Pro Asn Ala Tyr
                                                                                   480
                        150
                                                  155
145
                                                                           160
ttg gac gcg ttt gtg ttt atc ggc ggc gtc ggc gcg caa tac ggc gac
Leu Asp Ala Phe Val Phe Ile Gly Gly Val Gly Ala Gln Tyr Gly Asp
                                                                                   528
                   165
                                             170
acc gga cgg tgg att ttc gcc gct ggc gcg ttc gcg gca agc ctg atc
Thr Gly Arg Trp Ile Phe Ala Ala Gly Ala Phe Ala Ala Ser Leu Ile
                                                                                   576
              180
                                        185
tgg ttc ccg ctg gtg ggt ttc ggc gca gca gca ttg tca cgc ccg ctg
Trp Phe Pro Leu Val Gly Phe Gly Ala Ala Ala Leu Ser Arg Pro Leu
                                                                                   624
                                   200
                                                            205
tcc agc ccc aag gtg tgg cgc tgg atc aac gtc gtc gtg gca gtt gtg
Ser Ser Pro Lys Val Trp Arg Trp Ile Asn Val Val Val Ala Val Val
                                                                                   672
     210
                             215
                                                       220
atg acc gca ttg gcc atc aaa ctg atg ttg atg ggt tag
                                                                                  711
Met Thr Ala Leu Ala Ile Lys Leu Met Leu Met Gly
                         230
225
                                                  235
<210> 2
<211> 236
<212> PRT
<213> Brevibacterium lactofermentum
<400> 2
Met Val Ile Met Glu Ile Phe Ile Thr Gly Leu Leu Gly Ala Ser
                                              10
Leu Leu Leu Ser Ile Gly Pro Gln Asn Val Leu Val Ile Lys Gln Gly
Ile Lys Arg Glu Gly Leu Ile Ala Val Leu Leu Val Cys Leu Ile Ser
Asp Val Phe Leu Phe Ile Ala Gly Thr Leu Gly Val Asp Leu Leu Ser
                              55
Asn Ala Ala Pro Ile Val Leu Asp Ile Met Arg Trp Gly Gly Ile Ala
65 70 75 80
Tyr Leu Leu Trp Phe Ala Val Met Ala Ala Lys Asp Ala Met Thr Asn
85 90 95
Lys Val Glu Ala Pro Gln Ile Ile Glu Glu Thr Glu Pro Thr Val Pro
                                       105
                                                                 110
              100
Asp Asp Thr Pro Leu Gly Gly Ser Ala Val Ala Thr Asp Thr Arg Asn
                                                           125
         115
                                  120
Arg Val Arg Val Glu Val Ser Val Asp Lys Gln Arg Val Trp Val Lys
    130
                             135
                                                      140
Pro Met Leu Met Ala Ile Val Leu Thr Trp Leu Asn Pro Asn Ala Tyr
                        150
                                                  155
Leu Asp Ala Phe Val Phe Ile Gly Gly Val Gly Ala Gln Tyr Gly Asp
165 170 175
                   165
Thr Gly Arg Trp Ile Phe Ala Ala Gly Ala Phe Ala Ala Ser Leu Ile
180 185 190
Trp Phe Pro Leu Val Gly Phe Gly Ala Ala Ala Leu Ser Arg Pro Leu
                                   200
                                                            205
Ser Ser Pro Lys Val Trp Arg Trp Ile Asn Val Val Val Ala Val Val
    210
                             215
                                                      220
Met Thr Ala Leu Ala Ile Lys Leu Met Leu Met Gly
                        230
```

```
<210> 3
<211> 1197
<212> DNA
<213> Escherichia coli
<220>
<221> CDS
<222> (272)..(1153)
ccaggcgact gtcttcaata ttacagccgc aactactgac atgacgggtg atggtgttca 60
caattccacg gcgatcggca cccaacgcag tgatcaccag ataatgtgtt gcgatgacag
tgtcaaactg gttattcctt taaggggtga gttgttctta aggaaagcat aaaaaaaca 180
tgcatacaac aatcagaacg gttctgtctg cttgctttta atgccatacc aaacgtacca 240
ttgagacact tgtttgcaca gaggatggcc c atg ttc acg gga agt att gtc
                                                                                292
                                          Met Phe Thr Gly Ser Ile Val
gcg att gtt act ccg atg gat gaa aaa ggt aat gtc tgt cgg gct agc Ala Ile Val Thr Pro Met Asp Glu Lys Gly Asn Val Cys Arg Ala Ser 10 20
                                                                                340
ttg aaa aaa ctg att gat tat cat gtc gcc agc ggt act tcg gcg atc
Leu Lys Lys Leu Ile Asp Tyr His Val Ala Ser Gly Thr Ser Ala Ile
25 30 35
                                                                                388
gtt tct gtt ggc acc act ggc gag tcc gct acc tta aat cat gac gaa
Val Ser Val Gly Thr Thr Gly Glu Ser Ala Thr Leu Asn His Asp Glu
                                                                                436
                                                 50
 40
                         45
cat gct gat gtg gtg atg atg acg ctg gat ctg gct gat ggg cgc att
                                                                                484
His Āla Āsp Val Val Met Met Thr Leu Asp Leu Ala Āsp Glý Arg Ile
                    60
ccg gta att gcc ggg acc ggc gct aac gct act gcg gaa gcc att agc
Pro Val Ile Ala Gly Thr Gly Ala Asn Ala Thr Ala Glu Ala Ile Ser
                                                                85
ctg acg cag cgc ttc aat gac agt ggt atc gtc ggc tgc ctg acg gta
Leu Thr Gln Arg Phe Asn Asp Ser Gly Ile Val Gly Cys Leu Thr Val
                                                                                580
                                   95
                                                          100
                                                                                628
acc cct tac tac aat cgt ccg tcg caa gaa ggt ttg tat cag cat ttc
Thr Pro Tyr Tyr Asn Arg Pro Ser Gln Glu Gly Leu Tyr Gln His Phe
                                                     115
                             110
     105
                                                                                676
aaa gcc atc gct gag cat act gac ctg ccg caa att ctg tat aat gtg
Lys Ăla Ile Ăla Ğlu His Thr Ăsp Leu Pro Gln Ile Leu Tyr Asn Val
                                                130
                                                                        135
120
                        125
ccg tcc cgt act ggc tgc gat ctg ctc ccg gaa acg gtg ggc cgt ctg
                                                                                724
Pro Ser Arg Thr Gly Cys Asp Leu Leu Pro Glu Thr Val Gly Arg Leu
                   140
                                            145
gcg aaa gta aaa aat att atc gga atc aaa gag gca aca ggg aac tta
                                                                                772
Ala Lys Val Lys Asn Ile Ile Gly Ile Lys Glū Ala Thr Gly Asn Leu
              155
                                       160
acg cgt gta aac cag atc aaa gag ctg gtt tca gat gat ttt gtt ctg
Thr Arg Val Asn Gln Ile Lys Glu Leu Val Ser Asp Asp Phe Val Leu
                                                                                820
ctg agc ggc gat gat gcg agc gcg ctg gac ttc atg caa ttg ggc ggt
                                                                                868
    Ser Gly Asp Asp Ala Ser Ala Leu Asp Phe Met Gln Leu Gly Gly
                             190
                                                     195
                                                                                916
cat ggg gtt att tcc gtt acg act aac gtc gca gcg cgt gat atg gcc
```

```
US-102
His Gly Val Ile Ser Val Thr Thr Asn Val Ala Ala Arg Asp Met Ala
                      205
                                             210
                                                                    215
200
cag atg tgc aaa ctg gca gca gaa gaa cat ttt gcc gag gca cgc gtt
                                                                           964
Glň Meť Cýs Lys Leu Ála Ála Glu Glu His Phe Ála Glu Ala Arg Val
                  220
                                        225
att aat cag cgt ctg atg cca tta cac aac aaa cta ttt gtc gaa ccc
                                                                           1012
Ile Asn Glñ Arg Leu Met Pro Leu His Asn Lys Leu Phe Val Glu Pro
             235
                                    240
                                                                          1060
aat cca atc ccg gtg aaa tgg gca tgt aag gaa ctg ggt ctt gtg gcg
Asn Pro Ile Pro Val Lys Trp Ala Cys Lys Glu Leu Gly Leu Val Ala
acc gat acg ctg cgc ctg cca atg aca cca atc acc gac agt ggt cgt
Thr Asp Thr Leu Arg Leu Pro Met Thr Pro Ile Thr Asp Ser Gly Arg
                                                                          1108
                           270
    265
gag acg gtc aga gcg gcg ctt aag cat gcc ggt ttg ctg taa
Glu Thr Val Arg Ala Ala Leu Lys His Ala Gly Leu Leu
                                                                           1150
                      285
                                             290
                                                                          1197
agtttaggga gatttgatgg cttactctgt tcaaaagtcg cgcctgg
<210> 4
<211> 292
<212> PRT
<213> Escherichia coli
<400> 4
Met Phe Thr Gly Ser Ile Val Ala Ile Val Thr Pro Met Asp Glu Lys
                                         10
Gly Asn Val Cys Arg Ala Ser Leu Lys Lys Leu Ile Asp Tyr His Val
20 25 30
Ala Ser Gly Thr Ser Ala Ile Val Ser Val Gly Thr Thr Gly Glu Ser 40 45
Ala Thr Leu Asn His Asp Glu His Ala Asp Val Val Met Met Thr Leu
Asp Leu Ala Asp Gly Arg Ile Pro Val Ile Ala Gly Thr Gly Ala Asn 65 70 75 80
Ala Thr Ala Glu Ala Ile Ser Leu Thr Gln Arg Phe Asn Asp Ser Gly
                                         90
                   85
Ile Val Gly Cys Leu Thr Val Thr Pro Tyr Tyr Asn Arg Pro Ser Gln
100 105 110
                                                          110
Glu Gly Leu Tyr Gln His Phe Lys Ala Ile Ala Glu His Thr Asp Leu
115 120 125
Pro Gln Ile Leu Tyr Asn Val Pro Ser Arg Thr Gly Cys Asp Leu Leu
                                                 140
                          135
Pro Glu Thr Val Gly Arg Leu Ala Lys Val Lys Asn Ile Ile Gly Ile
145 150 155 160
Lys Glu Ala Thr Gly Asn Leu Thr Arg Val Asn Gln Ile Lys Glu Leu
                                        170
                                                               175
                  165
Val Ser Asp Asp Phe Val Leu Leu Ser Gly Asp Asp Ala Ser Ala Leu
                                    185
                                                           190
             180
Asp Phe Met Gln Leu Gly Gly His Gly Val Ile Ser Val Thr Thr Asn
                               200
                                                      205
Val Ala Ala Arg Asp Met Ala Gln Met Cys Lys Leu Ala Ala Glu Glu
                          215
    210
                                                 220
His Phe Ala Glu Ala Arg Val Ile Asn Gln Arg Leu Met Pro Leu His
225 230 235 240
```

```
US-102
Asn Lys Leu Phe Val Glu Pro Asn Pro Ile Pro Val Lys Trp Ala Cys
                                     250
                245
Lys Glu Leu Gly Leu Val Ala Thr Asp Thr Leu Arg Leu Pro Met Thr
                                 265
                                                      270
            260
Pro Ile Thr Asp Ser Gly Arg Glu Thr Val Arg Ala Ala Leu Lys His
        275
Ala Gly Leu Leu
    290
<210> 5
<211> 36
<212> DNA
<213> Artificial Sequence
<223> Description of Artificial Sequence: primer
cgggtggagg tgagcgtcgg taagcagcgg gtttgg
                                                                     36
<210> 6
<211> 33
<212> DNA
<213> Artificial Sequence
<223> Description of Artificial Sequence: primer
<400> 6
                                                                     33
gtctttttgt tcatcaccag caccttgggc gtt
<210> 7
<211> 39
<212> DNA
<213> Artificial Sequence
<223> Description of Artificial Sequence: primer
<400> 7
                                                                     39
agggaattcc ccgttctgga taatgttttt tgcgccgac
<210> 8
<211> 58
<212> DNA
<213> Artificial Sequence
<223> Description of Artificial Sequence: primer
                                                                     58
cggatgcatc tagagttaac ctgcagggtg aaattgttat ccgctcacaa ttccacac
<210> 9
<211> 64
```

US-102	
212> DNA 213> Artificial Sequence	
220> 223> Description of Artificial Sequence: primer	
400> 9 atttcctgc aggcaaagga gatgagcgta atggtgatca tggaaatctt cattacaggt 6 tgc	60 64
210> 10 211> 50 212> DNA 213> Artificial Sequence	
220> 223> Description of Artificial Sequence: primer	
400> 10 ggcgagcta gaagagctcc aaaacccgcg aaaactaacc catcaacatc 5	50
210> 11 211> 35 212> DNA 213> Artificial Sequence	
220> 223> Description of Artificial Sequence: primer	
400> 11 gacctgcag gtttgcacag aggatggccc atgtt	35
210> 12 211> 36 212> DNA 213> Artificial Sequence	
220> 223> Description of Artificial Sequence: primer	
400> 12 attctagat ccctaaactt tacagcaaac cggcat	36
210> 13 211> 30 212> DNA 213> Artificial Sequence	
220> 223> Description of Artificial Sequence: primer	
400> 13 cacggatca ctgtattcgg ctgcaacttt	30
210> 14	

US-102

<211> 32 <212> DNA <213> Artificial Sequence

<220> <223> Description of Artificial Sequence: primer

<400> 14 gccgtgttgc taggatggtt gttcttggat ca

32